A STUDY ON ACCURACY OF RESPIRATORY CYTOLOGY FOR CANCER DIAGNOSIS

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CERTIFICATE

This is to certify that this dissertation on **A STUDY ON ACCURACY OF RESPIRATORY CYTOLOGY FOR CANCER DIAGNOSIS** is a work done by **Dr.M.R.SUREKHA,** under my guidance during the period 2003-2006. This has been submitted in partial fulfillment of the award of M.D. Degree in Pathology (Branch-III) by the Tamil Nadu Dr.M.G.R. Medical University, Chennai.

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CONTENTS

INTRODUCTION

Sputum cytology has been the traditional focus for teaching respiratory cytology for many years. However the emphasis has been altered by the introduction of Fiberoptic bronchoscopy (FOB) and fine needle aspiration[22]. Recent developments in sampling techniques have changed the practice of respiratory tract cytology, although new methods have not completely supplanted more traditional ones. Methods for obtaining cell samples from the respiratory tract include sputum, bronchial brushing, bronchial washing, bronchioalveolar lavage, transbronchial needle aspiration, transthoracic fine needle aspiration and endoscopic ultrasonography guided fine needle aspiration. Each of these methods has advantages and limitations. Bronchial brushings, washing, fine needle aspiration and lavage procedures usually yield better diagnostic material than is obtained by simple exfoliative sampling[22].

Bronchial washing is complementary to brushing when an endobronchial lesion is observed and superior to brushing when the lesion is beyond the reach of the brush. It is also helpful in the diagnosis of peripheral lung lesions with submucosal or peribronchial tumour spread[18]. Washings are sent as part of the procedure and are routinely processed and add a small increment to sensitivity, mainly when brush or biopsy cannot reach more peripheral tumours[22].

Bronchioalveolar lavage is another technique particularly useful when a diffuse infiltrate is seen on the X-ray and an opportunistic infection or lymphangitic spread of tumour is suspected. The bronchioalveolar lavage may provide a higher yield than bronchial washing for diagnosis of peripheral tumours, particularly adenocarcinoma and bronchioloalveolar carcinoma.

The bronchoscopic approach to the diagnosis of tumours of the tracheobronchial tree is guided primarily by the size and location of the tumour. A combination of cytologic modalities is often performed with or without forceps biopsy to increase the diagnostic yield[18]. Central bronchogenic lesions may present as an exophytic mass, a submucosal or infiltration lesion or extrinsic bronchial compression and narrowing.

The use of fiberoptic bronchoscopic instruments and simultaneous recording of the findings on videotape for future review has significantly enlarged the ability to localise early lesions. Roughening and redness of the bronchial epithelium, especially in the areas of bronchial spurs and in areas of bronchial subdivisions, may signal an important lesion. Bronchial brushing of such areas for cytologic examination and biopsies of even tiny lesions are now technically feasible and have been successfully implemented[13].

While forceps biopsy is suitable for endobronchial mass lesions, bronchial brushing allows sampling of a larger mucosal area. If the mucosa appears grossly normal, however, little or no information may be obtained[18].

Combined study of cytology and biopsy material enhances the sensitivity of diagnosis of malignant tumours and their specific subtyping.

The combined use of cytology and biopsy facilities accurate classification of the tumour type, since cytologic samples often provide better morphologic preservation of the cells and lower likelihood of crushing artifacts (particularly in small cell carcinoma), whereas histologic samples better demonstrate tissue architecture and provide more material for ancillary techniques such as immuno histochemistry. Thus, even in the presence of an endobronchial lesion, collection of cytologic samples is recommended in addition to forceps biopsy.

AIM

Cytology is less invasive, more convenient and at times more accurate than tissue biopsy. In the experienced hands, cytology is highly reliable and can be used for definitive treatment without the need for further confirmatory tests.

The aim of the present study is to evaluate our institutional experience with bronchial wash, brush cytology and biopsy as diagnostic tools to enhance the sensitivity of diagnosis of malignant tumours.

However, the present study is designed to emphasize the diagnostic effectiveness of conventional respiratory cytologic methods and to advocate the combined use of fiberoptic biopsy in order to complement the cytologic diagnosis of lung cancer.

REVIEW OF LITERATURE

Respiratory cytodiagnosis had its birth in the late 1800s. In 1930s and 1940s, sputum cytology was a means of detecting or diagnosing lung cancer. The next decades brought expended use of this modality of cytodiagnosis and with it, more precise cytological subtyping of lung cancer and an evaluation of accuracy and clinical value[22].

George N. Papanicolaou and **Koprowska** were the first to report the cytological findings from the case of carcinoma in situ of the lung. The sputum cytological detection of early lung cancer and its precursors became the subject of attention during the 1960s and 1970s with the introduction and investigation of screening programmes for asymptomatic high risk groups, mainly cigarette smoking older males[22].

The development of the rigid bronchoscope in the late 19th century by **Gustav Killian**, formed the foundation of a technology by which the mucosal surface of the bronchi could be directly visualised and sampled for both tissue and cellular evaluation. Rigid bronchoscopic biopsy was the standard method of obtaining specimens for definitive diagnosis as a basis for management until the advent of flexible fibre optic bronchoscopy in the 1960s[4].

Dr.S.Ikeda, was the inventor of the Olympus Flexible Fiberoptic Bronchoscope. In 1964 Ikeda et al. developed standards for the flexible fiberoptic bronchoscope and in 1968, it was described as a diagnostic instrument.

The Bronchoscopist of today can perform laser therapy, cryotherapy, bradytherapy, stenting, localization of areas of dysplasia and carcinoma in situ using tissue autofluorescence and ultrasound localization of mediastenal nodes for transbronchial needle aspiration-all procedures that were unimagined or impossible 20 years ago!

By the 1980s fine needle aspiration cytology was established as having a pivotal role in the diagnosis and management of intrathoracic lesions. The last few decades have seen ample demonstration of the sensitivity and predictive value of cytodiagnosis as a basis for management, and gradual extension of the range of diagnosis to virtually all neoplastic processes affecting the lung and mediastenum[22].

Studies in the literature document the level of accuracy that may be achieved in the detection and classification of lung neoplasms through the use of sputum, bronchial washings and bronchial brushings.

Many publications reported new techniques, detection of neoplastic cells and cytohistologic correlation. The studies by Archer and colleagues, Wandall, Hampson, Bamforth, Grunze, Russell and associates, Woolner and coworkers, McDonald, Papanicolaou and colleagues, Farber and associates, Clerf and Harbut, Herbut, Foot, Umiker, Richardson and colleagues, Koss and coworkers, Spjut and coworkers, von Haam and others were significant contribution among these early investigations[4].

There is a great variation in the reported accuracy of bronchoscopic sampling methods. In a review of 21 papers published from 1970 to 1991, **Sing et al.** noted that the sensitivity of bronchial brushing ranged from 30% to 97.7% with an average of 65.9%. This wide range of accuracy reflects many factors including patient selection, different sampling devices, collection techniques, laboratory preparation methods, the expertise of the endoscopist and pathologist, all of which may influence the clinicians choice of bronchoscopic sampling modality. Most authors agree that the accuracy of lung cancer diagnosis is greatly improved when multiple sampling methods are employed.[18]

On average, each cytologic method detects about one-half to two-thirds of the lung cancers. Combining multiple methods results in a sensitivity of about 80% that is equal to or higher than that of bronchial forceps biopsy. Biopsy and cytology are complementary, however and by using both methods, a detection rate as high as 85% to 90% can be achieved.[18]

Zaharopoulos et al. discussed the cytology of small cell variants in detail. This is, however, a rare finding in routine cytological material[22].

Stuart Harris et al. described less than 2% of such tumours in routine diagnostic material from small cell cancers[22]. **Landsman** and his associates[4] compared the diagnostic accuracy of bronchial brushings and needle aspirates and found that brushings detected 89% of lung cancers whereas aspirates detected only 72%.

The importance of examining several specimens was also studied by **Erozan and Frost** in 1970. Among their patients with lung cancer, one bronchoscopic examination yielded diagnostic cytologic results in 61%. whereas one sputum specimen yielded diagnostic cytologic results in only 42%. Diagnostic yield however increased to 82% with three sputum examination and to 91% with five[4]. **Bedrossian** and his associates in 1976 reported a sensitivity of 56% in cancer detection when three sputum samples were examined. This rate increased to 76% when either bronchial brushings or bronchial washings were used. **Pilotti** and colleagues reported for sputum an overall sensitivity of 57% and for bronchial brushings at 67% rate. **Ng and Horak** reported in 1983 an overall sensitivity of 74% for bronchial washings and 83% for three sputum samples. **Ng** and **Horak**, in their bronchial washing study, reported that the accuracy of diagnosing tumour cell type was 96% for squamous cell carcinoma, 86% for adenocarcinoma, 77% for large cell carcinoma and less than 50% for Bronchioalveolar carcinoma. **Truong** and associates[4], in their 1985 study, determined that the overall sensitivities of sputum, bronchial washings and bronchial brushings were 60%, 66% and 77% respectively (Table 5). Their false-positive rate was 2.8%. **Tanaka** and associates examined the accuracy of cytologic diagnosis and typing in 154 patients. Central lesions were detected in 57% to 64% of the cases of either 3-day pooled sputum or aerosol induced specimens[4].

Sputum has shown the highest levels of sensitivity in detecting the more centrally located tumours, but this sensitivity has declined

drastically for the peripheral cancers. Bronchial brushing techniques for these peripheral lesions have improved diagnostic accuracy in cancer detection upto the levels of 70% to 88% of cases[4].

In 1973, **Bibbo** and associates reported 693 specimens obtained by fluoroscopically controlled bronchial brushing techniques. The series included 224 confirmed primary tumours and 30 metastatic tumours. For primary tumours, the average diagnostic yield (sensitivity) was 70% and 53% for metastatic lesions. In 160 cases, sputum samples taken before brushings showed tumour cells in only 7% of cases; however, sputum samples after brushing showed an increase to 66% tumour detection rate. Nine false-positive diagnosis were recorded and reported as a 2% rate[4]. **Bibbo** has emphasized the excellence of cellular preservation and the increased amounts of tumour cells arranged in irregular sheets as compared with sputum and bronchial washings.

In an extensive study of the results of pulmonary cytology emanating from the laboratory of **Koss, L.G.,** (**Koss et al.,** 1964), it was emphasized that careful collection and processing of material were essential in order to achieve satisfactory diagnostic results. Positive identification of lung cancer in an unselected series should be 60-70%. The accuracy of positive diagnosis may be increased by atleast 10% with three or more cytologic samples. With this number of samples, only about 10% of patients will fail to show any abnormal cells[13]. Thus, with perseverance a diagnostic accuracy of 90% is entirely possible[13]. The use of X-ray television and bronchial brushing, as originally suggested by

Hattori[13] and now widely accepted, or of direct aspiration of lung lesions, as discussed by **Dahlgren** and **Nordenstrom**, appears to increase substantially the yield of cytologic diagnosis of tumour located at the periphery of the lung. Diagnostic errors in the terms of positive cytologic diagnosis in the absence of cancer should not exceed 0.25% of cases.

In the study by **Koss et al.**[13] a comparison of bronchoscopic biopsies with cytology of bronchial aspirates was made (Table 1).

Table: 1

Comparison of patients with bronchoscopy and bronchial aspirates

Total No. of patients with bronchoscopy and bronchial aspirates	Aspirates positive	Biopsy positive
560	288 (40.7%)	117 (20.9%)

In 272 cases, no biopsy was obtained. The above results pertain to the use of a rigid bronchoscope and have now been superseded by brushing under roentgenologic guidance using a small radioopaque catheter and by fiberoptic bronchoscopy with brushing[13]. For example, **Solomon et al.** obtained positive cytologic identification by brushing in 41 of 46 patients with bronchogenic carcinoma[13].

Similarly rewarding results were recorded by **Skitarelic and von Haam** in a series of 204 consecutive cases. Bronchial brushing cytology

identified 85% of all cancers, when compared with 76% for sputum and 81% for bronchial washings[13].

Bibbo et al. (1973) studied 224 patients with primary peripheral bronchogenic carcinoma. The diagnostic yield of brushing varied from 60% for adenocarcinoma to 81% for squamous cell carcinoma. Comparison of the yield of bronchial brushing with that of sputum in 160 cases revealed positive sputum prior to brushing on only 27 patients when compared with 106 positive diagnosis obtained by brushing. It is of interest that after brushing, an additional 28 patients had positive sputum. Thus, the superiority of brushing when compared with sputum for the diagnosis of peripheral lung lesions, has been firmly established, as originally advocated by **Hattori**[13].

Epidemiology

Lung cancer was a rare disease until the early 1900s, but is now the most common cancer in the United States and worldwide. Lung cancer, is by far, the leading fatal cancer in both men (31%) and women (25%) compared to prostate (10%), colon and rectum (10%) in men and 11% in women and breast carcinoma (15%)[18].

Etiology

The causes of lung cancer can be divided into genetic and environmental. The increased incidence in the 20th century followed the

explosive growth of cigarette smoking. Cigarette smoke contains irritants, oxidants, free radicals, carcinogens and a variety of toxins. In smokers, with asbestos exposure the lung cancer rate is approximately 50 times that of non exposed individuals.

Although cigarette smoking accounts for the majority of the cancers, a proportion (9-15%) in various studies has been attributed to occupational exposures. One of the most common, asbestos, is a group of naturally occurring fibrous materials. Since the 1950s, numerous epidemiologic studies have established that asbestos exposure independently increases the risk of lung cancer.

Radon is an inert radioactive gas produced by the natural decay of radium. It is present in most soils and rocks in various concentrations. Epidemiologic studies on underground miners have established a causal relationship to lung cancer[18].

Sampling and cytopreparatory techniques

The diagnostic accuracy of cytology begins with a foundation of excellence in cytopreparation of these specimens. A respiratory tract specimen that has been prepared for cytologic examination, should exhibit an abundance of well preserved and stained diagnostic material. It should have been prepared rapidly, with relative care and should survive permanent slide storage.[4]

Bronchial aspirates and washings

Introduction of the bronchoscope in the lower respiratory tract enables the examiner to obtain specimens by means of a suction apparatus that aspirates secretion. Washings from the visualized areas may also be collected by instilling 3-5ml of a balanced salt solution through the bronchoscope and re-aspiration of the resulting material. Once the bronchoscope is removed, direct smears may be made with immediate fixation in 95% ethyl alcohol.

Bronchial wash has a lower diagnostic yield than bronchial brushing. However it is important for diagnosis of peripheral lesions, infections and bronchioloalveolar carcinoma[4].

Bronchial brushings

By using flexible fiberoptic bronchoscope it is possible to visualise and brush a suspected lesion and submit the resulting cytologic material for laboratory examination[4.]

Bronchioalveolar lavage

This involves the infusion and re-aspiration of a sterile saline solution in distal segments of the lung via a fiberoptic bronchoscope. The most important diagnostic application of BAL is for detecting opportunistic infection in immunocompromised hosts.

Fine needle aspiration

In this procedure, a fine needle attached to a syringe is passed through the chest wall or bronchial wall into the pulmonary mass visualized by fluroscopy, computed tomography or bronchoscopy. The aspirated cellular specimen is examined by conventional cellular techniques.

Recalling the histogenesis of primary lung cancers, is very persuasive as an aid in comprehending exactly why it is that cytologic diagnosis of the respiratory tract has been so successful. It is mainly because most primary lung cancers arise from the epithelium lining the respiratory passages and have the potential of shedding cancer cells into specimens of sputum or of having their cells harvested for cytologic diagnosis by methods of fiberoptic bronchoscopy, bronchioalveolar lavage or fine needle aspiration[4].

Ancillary techniques

Cell blocks can be prepared by several techniques. Sputum cell blocks may be of value for the diagnosis of carcinoma. Cell blocks on FNA samples are most easily prepared by using powdered thrombin to induce clotting in a slide or watch glass and by fixing and processing the resulting pellet as for biopsy material, so removing washing or centrifugation steps.

Cell blocks are useful for `microhistology' to detect architectural features not evident in smears and for cytochemistry using mucin stains.

Advantages of bronchoscopy

1. Accurate localization of tumors within the reach of the bronchoscope.
2. Accurate diagnosis of tumour type by means of a bronchoscopic biopsy.
3. Estimation of the spread of the tumour within the bronchial tree.
4. Additional information may be obtained if conventional cytology and bronchial brushing are combined with bronchoscopy[13].

Disadvantages of bronchoscopy

1. The procedure is time consuming
2. It is quite unpleasant to the patient and carries with it some morbidity.
3. The area of sampling is limited.
4. It is not suitable as a procedure for mass screening for lung cancer[13].

Complications of FOB

1. Bronchoscopy is avoided in patients with moderate to severe coagulation disorders.

2. Pneumothorax, hemoptysis and significant bronchospasm-very rare

3. Mortality-extremely low.

BRONCHIAL WASHING

Advantage

It is possible to sample extensive portions of the bronchial tree.

Disadvantage

Blood, debris and inflammatory cells can obscure the diagnostic cells.

BRONCHIAL BRUSHING

Advantages

1. Easier visualisation of the lesion

2. Fresh cells can be obtained

Disadvantages

1. Limited sampling of the bronchial tree

2. Must be experienced to accurately sample the lesion

Processing of Exfoliative cytology specimens

Proper collection, fixation and optimal processing of respiratory cytology specimens is critical. Sputum may be processed as a direct

smear and/or cytospin/liquid based cytology smear. A direct smear can be made and wet fixed in 95% ethanol. Bronchial brushes are smeared directly on the slide and wet fixed immediately in alcohol[2].

If special stains are needed, additional cytospin smears or liquid-based cytology smears may be prepared. Ancillary studies, such as cell block, flow cytometry and electron microscopy can be performed on fresh unfixed lavage and wash specimens[2].

Interpretation of Exfoliative respiratory cytology

During the interpretation of exfoliative cytology, an essential assessment is adequacy of the specimen. Presence of alveolar macrophages in sputum smears represents an adequate sample. For bronchial brushing and wash, ciliated columnar cells (6-10 groups or sheets with multiple single cells), a few mucous goblet cells and alveolar macrophages should be identified[2]. Adequate bronchoalveolar lavage should show numerous alveolar macrophages with a few lymphocytes. If these features are not found, the specimen should be categorized as `inadequate, consistent with sampling artifact' to communicate its non representative nature. Large numbers of oral squamous cells, extensive crush artifact, poor preservation, saprophytic organisms such as Candida and Actinomyces and significant air-drying artifacts compromising the interpretation should not be present[2].

In general the cytomorphologic features of lung carcinoma in exfoliative cytology specimens are similar to those of FNAB cytology[2]. However, a few differences do exist. Single cells & degenerative changes are more frequent in spontaneously exfoliated specimens (sputum) compared with mechanically exfoliated material (bronchial brushing, bronchial wash & BAL). Squamous cell dysplasia[2] and carcinoma-in-situ cannot be diagnosed by FNAB, however they can sometimes be observed in exfoliative cytology specimens like brushing. These lesions should be differentiated from squamous metaplasia. Squamous cell dysplasia and carcinoma-in-situ are usually seen as small groups or single cells with large, irregular and hyperchromatic nuclei without tumour diathesis[2].

Normal and reactive cells in exfoliative cytologic specimens

Various types of normal and reactive cells seen in the cytology specimens include-mature squamous cells, squamous metaplastic cells, ciliated columnar cells, mucous goblet cells, basal reserve cells, Clara cells, type 1 and type 2 pneumocytes, macrophages and inflammatory cells.

Squamous cell carcinoma has variable morphology in cytologic samples, depending on the degree of tumour differentiation, collection method and preparation techniques. In general, tumour cells appear singly or in small groups in exfoliative cytology (i.e. sputum, bronchial washing) whereas in bronchial brushings and needle aspirates, larger tissue fragments are present in addition to single cells. Loss of

cohesiveness is more pronounced in well-differentiated than in poorly differentiated tumours. Thus the former presents with single tumour cells and the latter sheds large cell clusters.[18] **Well differentiated, keratinizing squamous cell carcinoma** is characterised by the presence of large pink and orangiophilic cells that exhibit marked variation in size and shape. Long slender tadpole shaped, angulated and irregular `fibre' cells are frequently seen. Significant anisonucleosis and pleomorphism are common. The cytoplasm is dense and nuclei are hyperchromatic with irregularity of nuclear membrane. Nucleoli are present, but not prominent. Squamous pearls composed of concentric clusters of elongated eosinophilic cells with hyperchromatic nuclei are characteristic of this tumour.

Poorly differentiated squamous cell carcinoma is characterized by malignant cells that are generally smaller than the well differentiated variant and exhibit more basophilic cytoplasm. The nuclei have coarse chromatin and nuclear cytoplasmic (N/C) ratio is high.[18]

Bronchioalveolar adenocarcinoma is composed of several cell types that are usually pure. These are mucus producing carcinomas that contain mucin filled cells and non-mucinous tumours are composed of either Type 2 pneumocytes, Clara cells or combination of the two. There is a wide range of cellular differentiation but usually the tumours are composed of cells which have small nuclei, cellular uniformity and little mitotic activity. Necrosis is usually absent.[18]

Small cell carcinoma

Poorly differentiated small cell carcinoma demonstrates numerous syncytial groups of hyperchromatic cells with single cells. Most of the cells are small and are usually less than the diameter of three small lymphocytes. The apoptotic cells are frequent. This produces a dimorphic population with mixture of viable and non-viable cells. The nuclei are usually round to oval, but they may be irregular and demonstrate molding. Nuclear molding should be differentiated from the subtle adjustment of nuclear shapes associated with cellular molding[2]. The tumour cells have an extremely high nuclear to cytoplasm ratio with a scant amount of indistinct surrounding cytoplasm. Nuclear chromatin is finely granular with clumping and parachromatin clearing leading to a mixture of fine and coarse dots described as a **`salt and pepper'** chromatin pattern. The background of small cell carcinoma shows extensive necrotic debris and strands of basophilic material. The latter represent extended and smudged DNA from ruptured fragile nuclei. This `crush artifact' is best seen in aspirates and is produced while spreading the FNAB material between two slides. This is equivalent to **Azzopardis'** effect observed in surgical pathology material. Small foci of squamous or glandular differentiation may also be present in small cell carcinoma[2].

Metastatic carcinoma

Because most of the metastatic lesions in the lung do not communicate with the bronchial lumen and do not exfoliate diagnostic cells into the airway, exfoliative cytology is rarely useful. Usually, FNAB is the method of choice in such cases. However, metastases projecting as endobronchial lesion do occur rarely. Renal cell carcinoma (and other genitourinary tract carcinoma), breast carcinoma and malignant melanoma are the most common metastatic lesions in this category. Large tissue fragments in exfoliative cytology specimens favour the possibility of a metastatic tumor[2].

Occasionally the cytomorphology may be remarkable without any resemblance to usual primary lung carcinomas. However, generally the cytomorphologic features overlap with the adenocarcinoma of lung. Thus, in most of the cases, metastasis may be difficult to interpret solely by cytomorphology without clinical study of primary tumour and application of ancillary tests such as immuno cytochemistry. A clinical history of known extrapulmonary malignancy and radiologic evidence such as multiple nodules consistent with metastasis is usually helpful[2].

Pitfalls in Respiratory Cytopathology

Cytomorphologically, many non-neoplastic lung lesions may simulate malignancy and are potential pitfalls leading to malignant misinterpretation. Proper correlation with clinical and radiologic features is essential.

Problems in diagnosis appear to arise primarily in bronchial washing specimens where tumour cells are smaller, limited in number and show extensive degeneration when compared to other respiratory tract specimens. Brushing and aspiration specimens tend to be very cellular with a dirty background, comprising debris from cytoplasm stripped during smearing.

Any infectious or inflammatory process may be associated with inflammatory atypia of epithelial cells, fibroblasts and histiocytes as a potential cause of a **False-positive** diagnosis of malignancy.

Degenerating histiocytes may have atypical features, including nuclear hyperchromasia and nucleoli. The vacuolated cytoplasm of histiocytes, simulates an Adenocarcinoma. Bronchioloalveolar carcinoma is the most common malignancy confused with atypical degenerating histiocytes and pneumocytes. Metaplastic cells in cavitary lesions like tuberculosis, lung abscess and aspergilloma can also be mistaken for malignant cells.

Frequently, patients who have had prior irradiation and/or chemotherapy have a lung aspiration performed to evaluate a new lesion. A **false positive** diagnosis of malignancy is possible in these patients because of the presence of atypical cells either of pulmonary epithelial origin or from the mesothelium[2].

Helpful features to suggest a correct diagnosis of chemotherapeutic or irradiation changes include atypical cells with cytomegaly without increase in nuclear to cytoplasmic ratio and tendency for multinucleation. The large, hyperchromatic, irregular nuclei show degenerative changes with smudged chromatin[2].

Granulomatous lesions show cohesive clumps of epithelioid histiocytes admixed with small lymphocytes. The histiocytes show relatively abundant amphophilic cytoplasm usually with indistinct cell margins imparting a syncytial appearance. These groups may be confused as epithelial cells and misinterpreted as carcinoma. Necrotizing granulomas containing tight clusters of epithelioid cells and necrotic debris on the background may be misinterpreted as carcinoma with tumour diathesis. Identification of bland nuclei and lack of true epithelial structures should prevent this pitfall[2].

Traditionally, reserve cell hyperplasia represents the major differential diagnostic consideration in these specimens. When disturbed during bronchoscopy procedures, reserve cells are shed as small cohesive fragments in contrast to the cells of small cell undifferentiated carcinoma, which remain only loosely aggregated. Although both reserve cells and cells of small cell undifferentiated carcinoma are small with hyperchromatic nuclei, reserve cells are uniform with smooth nuclear membranes and evenly distributed chromatin. Cells of small cell carcinoma remain as single cells or small clusters of cells with prominent nuclear molding and tumor diathesis.

Predominance of scattered single cells of small cell carcinoma may resemble lymphocytes and erroneously suggest a lymphoproliferative lesion, especially in liquid -based cytologic smears. Diff-Quik stained smears are invaluable for evaluating the nuclei of hematopoietic cells and observing lymphoglandular bodies. A monomorphic population of lymphocytes favours the diagnosis of Lymphoma. It is important not to misinterpret scattered single cells of small cell carcinoma as monomorphic lymphoid cells[2].

MATERIALS AND METHODS

We have studied 122 samples from the pulmonology department of Government Hospital of Thoracic Medicine at Tambaram and Otteri for a period of 3 years from Jan. 2003 to Dec. 2005. There was no age restriction. The age of the patients ranged from 20 to 80 yrs. The bronchial washings, brushings and biopsy samples were obtained from the patients with the help of the Flexible Fiberoptic Bronchoscope. Bronchial washing, brushing and biopsy samples were submitted simultaneously to our laboratory. The cytology samples were sent as unstained smears and the slides were stained with the standard Haemotoxylin and Eosin stain. They were examined on the same day without any knowledge about the bronchial biopsy specimens. The biopsy specimens were fixed in Neutral buffered formaldehyde, processed to paraffin blocks and also stained with Haematoxylin and Eosin stain. The slides were examined a few days following the cytologic examination. Clinical data and bronchoscopic findings were provided to the pathologist for some cases, while others were not accompanied by relevant clinical information. The cytology diagnosis was known at the time of examination of the biopsy specimens.

The cytology slides were examined by a skilled and experienced pathologist and the bronchial biopsy slides were viewed by another experienced pathologist.

Standard Haematoxylin and eosin stain for cytology and paraffin sections[10]

Method

1. The sections are deparaffinized and hydrated through graded alcohols to water.
2. Stain with Alum hematoxylin of choice.
3. Wash well in running tap water until `blueing' takes place-for 5 min.
4. Differentiate in 1% acid alcohol (1% Hcl in 70% alcohol) for 5-10 sec.
5. Wash well in tap water until sections are again blue.
6. Stain in 1% eosin Y for 10 min.
7. Wash in running tap water for 1-5 min.
8. Dehydrate through alcohols, clear and mount

Diagnostic categories used in reporting cytology findings included.

i. Positive for malignancy

ii. Negative for malignancy

iii. Atypical cells seen, suspicious for malignancy.

RESULTS

A total of 122 cases were studied from pulmonology department at Tambaram and Otteri. Among them 76.2% were males (Fig.1).

Age of the patients ranged from 20 to 80 yrs. The results of bronchial wash, brushing and biopsy samples are given (Table 2).

OBSERVATIONS FROM THE STUDY

Sl. No.	Patient name	Brush cytology	Bronchial biopsy	Result category
1.	Mani	p	p	TP
2.	Nagappan	n	n	TN
3.	Raju	n	n	TN
4.	Manickam	n	n	TN
5.	Palani	n	n	TN
6.	Vasanthy	n	n	TN
7.	Rajammal	p	p	TP
8.	Kanniappan	n	n	TN
9.	Balasubramanian	n	n	TN
10.	Kotteeswaramma	n	n	TN
11.	Devaraj	n	n	TN
12.	Mohanammal	n	n	TN
13.	Duraipandi	n	n	TN
14.	Sudakar	n	n	TN
15.	Gunaseelam	n	n	TN
16.	Kollapuri	p	p	TP
17.	Malarkodi	n	n	TN
18.	Raja	n	n	TN
19.	Shanmugam	n	p	FN
20.	Jayaraman	n	n	TN
21.	Usman	n	p	FN

22.	Raja	p	p	TP
23.	Nagaraj	n	n	TN
24.	Janakiraman	n	n	TN
25.	Premkumar	n	n	TN
26.	Munusamy	p	p	TP
27.	Samy	p	p	TP
28.	Saravanan	n	n	TN
29.	Ranganayagi	p	n	FP
30.	Poongavanam	p	p	TP
31.	Mannankath	p	p	TP
32.	Kalanjiammal	n	n	TN
33.	Kadirvel	p	p	TP
34.	Thangavel	n	n	TN
35.	Panchavarnam	n	p	FN
36.	Murugaiah	n	n	TN
37.	Moorthy	n	n	TN
38.	Perumal	p	p	TP
39.	Durairaj	p	p	TP
40.	Devandran	p	p	TP
41.	Rani	n	n	TN
42.	Pandian	p	p	TP
43.	Seenu	n	n	TN
44.	Seshammal	n	p	FN
45.	Masilamani	n	n	TN

46.	Ilavalli	n	n	TN
47.	Ramar	n	n	TN
48.	Nagappan	n	p	FN
49.	Thanthoni	p	p	TP
50.	Jagadeesan	p	p	TP
51.	Muthukaruppan	p	p	TP
52.	Manickam	p	p	TP
53.	Saradammal	p	p	TP
54.	Arumugam	p	p	TP
55.	Narasimman	p	p	TP
56.	Elumalai	p	p	TP
57.	Subramaniyan	p	p	TP
58.	Kanagavalli	p	p	TP
59.	Shakuntala	p	n	FP
60.	Sundaram	p	p	TP
61.	Munusamy	p	p	TP
62.	Arumugam	p	p	TP
63.	Anirunisha	n	n	TN
64.	Kanniappan	p	p	TP
65.	Subramani	p	p	TP
66.	Mabeeza	p	p	TP
67.	Vasantha	n	n	TN
68.	Perumal	p	p	TP
69.	Durai	p	p	TP

70.	Rangaiah	p	p	TP
71.	Periyasamy	p	p	TP
72.	Ellammal	p	p	TP
73.	Suseela	p	p	TP
74.	Kamalam	p	p	TP
75.	Arasan	p	p	TP
76.	Pandiyan	n	p	FN
77.	Narayanan	n	n	TN
78.	Nagappan	p	p	TP
79.	Muniammal	p	p	TP
80.	Amanullah	p	p	TP
81.	Karuppusamy	n	n	TN
82.	Chinnathambi	p	p	TP
83.	Ekambaram	p	p	TP
84.	Murugesan	n	n	TN
85.	Dubier	p	p	TP
86.	Devasigamani	p	p	TP
87.	Perumal	n	n	TN
88.	Michael	p	p	TP
89.	Narayanan	p	p	TP
90.	Chockalingam	n	n	TN
91.	Dhanapal	p	p	TP
92.	Mani	p	p	TP
93.	Murugesan	p	p	TP

94.	Santhiyagu	p	p	TP
95.	Sarojammal	p	p	TP
96.	Priya	p	p	TP
97.	Samikannu	p	p	TP
98.	Mariammal	p	p	TP
99.	Ayyakannu	p	p	TP
100.	Sabapathy	p	n	FP
101.	Kanagavalli	p	p	TP
102.	Banumathy	n	p	FN
103.	Elaghavan	p	p	TP
104.	Mangai	n	p	FN
105.	Velarasu	n	p	FN
106.	Palanivel	n	p	FN
107.	Rajammal	n	p	FN
108.	Venkaiah	p	p	TP
109.	Govindaraj	p	p	TP
110.	Jayachandran	n	p	FN
111.	Narayana Raj	n	p	FN
112.	Prabhakar	n	p	FN
113.	Mariammal	p	p	TP
114.	Mohamed Masthan	p	p	TP
115.	Kannaiyan	p	p	TP
116.	Gopal	p	p	TP
117.	Dhanapal	p	n	FP

118.	Dhanavel	p	p	TP
119.	Kondamma	p	p	TP
120.	Raja	p	p	TP
121.	Sundaram	p	p	TP
122.	Elumalai	p	p	TP

p - positive

n - negative

TP - True positive

TN - True negative

FP - False positive

FN -False negative

RESULTS OF TRUE POSITIVE CASES

Sl. No.	Patient name	Bronchial cytology	Bronchial biopsy
1	Mani	p	p
7	Rajammal	p	p
16	Kollapuri	p	p
22	Raja	p	p
26	Munusamy	p	p
27	Samy	p	p
30	Poongavanam	p	p
31	Mannankath	p	p
33	Kadirvel	p	p
38	Perumal	p	p
39	Durairaj	p	p
40	Devandran	p	p
42	Pandian	p	p
49	Thanthoni	p	p
50	Jagadeesan	p	p
51	Muthukaruppan	p	p
52	Manickam	p	p
53	Saradammal	p	p
54	Arumugam	p	p
55	Narasimman	p	p
56	Elumalai	p	p
57	Subramaniayan	p	p
59	Kanagavalli	p	p
60	Sundaram	p	p
61	Munusamy	p	p
62	Arumugam	p	p
64	Kanniappan	p	p

65	Subramani	p	p
66	Mabeeza	p	p
68	Perumal	p	p
69	Durai	p	p
70	Rangaiah	p	p
71	Periyasamy	p	p
72	Ellammal	p	p
73	Suseela	p	p
74	Kamalam	p	p
75	Arasan	p	p
78	Nagappan	p	p
79	Muniammal	p	p
80	Amanullah	p	p
82	Chinnathambi	p	p
83	Ekambaram	p	p
85	Dubier	p	p
86	Devasigamani	p	p
88	Michael	p	p
89	Narayanan	p	p
91	Dhanapal	p	p
92	Mani	p	p
93	Murugesan	p	p
94	Santhiyagu	p	p
95	Sarojammal	p	p
96	Priya	p	p
97	Samikannu	p	p
98	Mariamma	p	p
99	Ayyakannu	p	p
101	Kanagavalli	p	p
103	Elaghavan	p	p
108	Venkaiah	p	p

109	Govindaraj	p	p
113	Mariammal	p	p
114	Mohamed Masthan	p	p
115	Kannaiyan	p	p
116	Gopal	p	p
118	Dhanavel	p	p
119	Kondama	p	p
120	Raja	p	p
121	Sundaram	p	p
122	Elumalai	p	p

p - positive

RESULTS OF TRUE NEGATIVE CASES

Sl. No.	Patient name	Bronchial cytology	Bronchial biopsy
2	Nagappan	n	n
3	Raju	n	n
4	Manickam	n	n
5	Palani	n	n
6	Vasanthy	n	n
8	Kanniappan	n	n
9	Balasubramanian	n	n
10	Kotteeswarama	n	n
11	Devaraj	n	n
12	Mohanammal	n	n
13	Duraipandi	n	n
14	Sudakar	n	n
15	Gunaseelam	n	n
17	Malarkodi	n	n
18	Raja	n	n
20	Jayaraman	n	n
23	Nagaraj	n	n
24	Janakiraman	n	n
25	Premkumar	n	n
28	Saravanan	n	n
32	Kalanjiammal	n	n
34	Thangavel	n	n
36	Murugaiah	n	n
37	Moorthy	n	n
41	Rani	n	n
43	Seenu	n	n
45	Masilamani	n	n

46	Ilavalli	n	n
47	Ramar	n	n
63	Anirunisha	n	n
67	Vasantha	n	n
77	Narayanan	n	n
81	Karuppusamy	n	n
84	Murugesan	n	n
87	Perumal	n	n
90	Chockalingam	n	n

n - negative

RESULTS OF FALSE, NEGATIVE CASES

Sl. No.	Patient name	Bronchial cytology	Bronchial biopsy
19	Shanmugam	n	p
21	Usman	n	p
35	Panchavarnam	n	p
44	Seshammal	n	p
48	Nagappan	n	p
76	Pandiyan	n	p
102	Banumathy	n	p
104	Mangai	n	p
105	Velarasu	n	p
106	Palanivel	n	p
107	Rajammal	n	p
110	Jayachandran	n	p
111	Narayana Raj	n	p
112	Prabhakar	n	p

RESULTS OF FALSE POSITIVE CASES

Sl. No.	Patient name	Bronchial cytology	Bronchial biopsy
29	Ranganayagi	p	n
100	Sabapathy	p	n
117	Dhanapal	p	n
59	Shakuntala	p	n

DISCUSSION

In our present study, bronchial washings, brushings and biopsy specimens were obtained via fibreoptic bronchoscopy. In a total of 122 cases studied, 68 cases (55.73%) were diagnosed as `positive for malignancy' by cytology as well as by biopsy. Bronchial biopsy was taken as the gold standard for the study. The accuracy of bronchial wash and brush test in this study was found to be 85.24%. Majority of the patients in our study diagnosed as malignancy (30.3%) were in the age group of 50-60 years (Fig.2).

I.	No. of True Positive Cases	:	68
II.	No. of True Negative Cases	:	36
III.	No. of False Negative Cases	:	14
IV.	No. of False positive Cases	:	4

The sensitivity, specificity, accuracy, positive and negative predictive valve of Brush cytology were calculated relative to the final Histopathologic status as follows

$$\text{Sensitivity}^{19} = \frac{TP}{TP+FN}$$

$$\text{Specificity}^{19} = \frac{TN}{TN+FP}$$

$$\text{Accuracy}^{19} = \frac{TP+TN}{TP+FP+FN+TN}$$

$$\text{Positive predictive value}^{19} = \frac{TP}{TP+FP}$$

$$\text{Negative predictive value}^{19} = \frac{TN}{TN+FN}$$

Table: 3

Comparison of the results of Bronchial cytology and Biopsy

Bronchial cytology	**Bronchial Biopsy (Histopathology)**		**Total**
	Positive cases	**Negative cases**	
Positive	68	4	72
Negative	14	36	50
Total	82	40	122

Based on the above 2x2 table, the results were completed as follows:

A. Sensitivity - 82.92%

B. Specificity - 90%

C. Positive predictive value - 94.44%

D. Negative predictive value- 72%

E. Accuracy - 85.24%

The **sensitivity** and **specificity** are important factors in deciding the accuracy of the diagnostic test. The sensitivity of Bronchial cytology in our study is 82.9% whereas in other studies it ranges from 38 to 96%. The negative predictive value was 72%. The number of false negative cases was 14. False negative diagnosis is usually a result of sampling error and rarely due to interpretation error. The sampling error could probably be due to inaccessibility of the bronchial brush to the site of lesion or to faulty techniques of smearing on the slides.

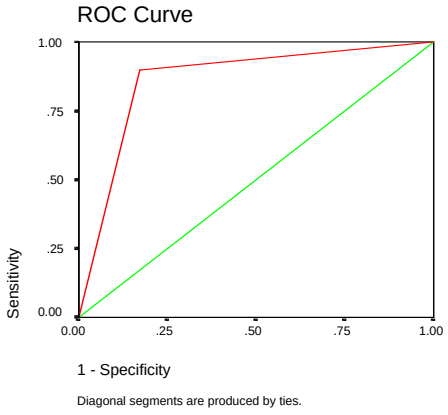
ROC Curve

Case processing summary

Bronchial biopsy	Valid N (list wise)
Positive[a]	40
Negative	82

Larger values of the test result variable(s) indicate stronger evidence for a positive actual state

a. The positive actual state is negative



Area under the curve

The result variable(s): Brush cytology

Area = .865

The test result variable(s): Brush Cytology has atleast one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

The difference in age distribution by bronchial biopsy/cytology is statistically significant (Table 4).

CROSSTABS

Table: 4

Age and Bronchial biopsy

Age		**Bronchial biopsy**		**Total**
		Positive	**Negative**	
>30 Years	Count % of total	1 (0.8%)	8 (6.6%)	9 (7.4%)
30-40 Years	Count % of total	9 (7.4%)	7 (5.7%)	16 (13.1%)
40-50 Years	Count % of total	21 (17.2%)	12 (9.8%)	33 (27.0%)
50-60 Years	Count % of total	29 (23.8%)	8 (6.6%)	37 (30.3%)
60-70 Years	Count % of total	17 (13.9%)	4 (3.3%)	21 (17.2%)
70-80 Years	Count % of total	5 (4.1%)	1 (0.8%)	6 (4.9%)

P<0.01 SS (99%)

Age and Bronchial Cytology

Age		Bronchial biopsy		Total
		Positive	Negative	
>30 Years	Count % of total	1 (0.8%)	8 (6.6%)	9 (7.4%)
30-40 Years	Count % of total	9 (7.4%)	7 (5.7%)	16 (13.1%)
40-50 Years	Count % of total	21 (17.2%)	12 (9.8%)	33 (27.0%)
50-60 Years	Count % of total	29 (23.8%)	8 (6.6%)	37 (30.3%)
60-70 Years	Count % of total	17 (13.9%)	4 (3.3%)	21 (17.2%)
70-80 Years	Count % of total	5 (4.1%)	1 (0.8%)	6 (4.9%)

P<0.01 SS (99%)

Accuracy is to a great extent influenced by the expertise of the aspirator and the pathologist as well as the methodology used to prepare the sample in the laboratory. The size and location of the tumour also have a significant impact on the success rate of the test. The accuracy of the test in this study is 85.24% which is in the reference range of the most widely acclaimed studies published earlier (75-99%) (Table 5).

Table: 5

Sensitivity and specificity of Bronchial wash/brush in various studies

Investigator	Year	Specimen type	Sensitivity (%)	Specificity (%)
Erosan and Frost	1970	Bronchial washing	61	NS
Bibbo et al.	1973	Bronchial brushing	70	98
Bedrossian et al.	1976	Bronchial washing Bronchial brushing	76 76	NS
Johnston and Bossen	1981	Bronchial wash Bronchial brush	22 87	99.9
Pilotti et al.	1982	Bronchial brush	67	NS
Ng and Horak	1983	Bronchial washing	74	NS
Truong et al.	1985	Bronchial washing Bronchial Brushing	66 77	99.9
Present study	2005	Bronchial washing Bronchial brushing Bronchial biopsy	82.9	90

NS - Not supplied

POSSIBLE PITFALLS IN THIS STUDY

1. The size of the sample is small to statistically signify the high false negative rate.

2. Our study was a blind study. Review of the cytodiagnosis following histopathology diagnosis could have increased the accuracy rate.

3. 27 cases that were diagnosed as `positive for malignancy' by brush cytology were not subjected to bronchial biopsy. Hence they were not included in the study. If these cases had histopathological confirmation, that would have increased the overall diagnostic yield.

4. 20 cases were not subjected to cytological examination (bronchial washing or brushing) but were directly subjected to biopsy and diagnosed as positive for malignancy. If cytology was done in these cases, the sensitivity would have been further increased.

5. In our present study, there were four false positive cases. Multiple cytology sampling could have been done to minimize the false positive rate.

6. False negativity could have been due to sampling error and poor fixation.

CONCLUSION

Our study suggests that the accuracy of Bronchial cytology (brushing and washing) is high enough to warrant its use in combination with bronchial biopsy in the diagnosis of lung cancer.

Cytological procedures of bronchial washing and brushing yield acceptable optimum results in case of peripheral lesions even in absence of fluoroscopically guided bronchoscopy.

There are situations in which the cytologic-histologic correlation is not high. In such cases, it should not be concluded that the cytologic interpretation is obviously an error and that the histologic interpretation is correct. Although this was the original thesis on which the discipline of cytology was founded, cytology has matured and come of age, and it can now be appreciated that in some situations the cytologic interpretation may be just as correct as the tissue interpretation and in some cases, more accurately reflective of the nature of the lesion than the tissue examined[4].

Though the sensitivity of brush cytology is high, further evaluation by histopathological examination using traditional staining technique such a H&E still remains the gold standard and is still indicated.

The correlation between cytologic and histological diagnosis is excellent in well differentiated squamous cell carcinoma, adenocarcinoma

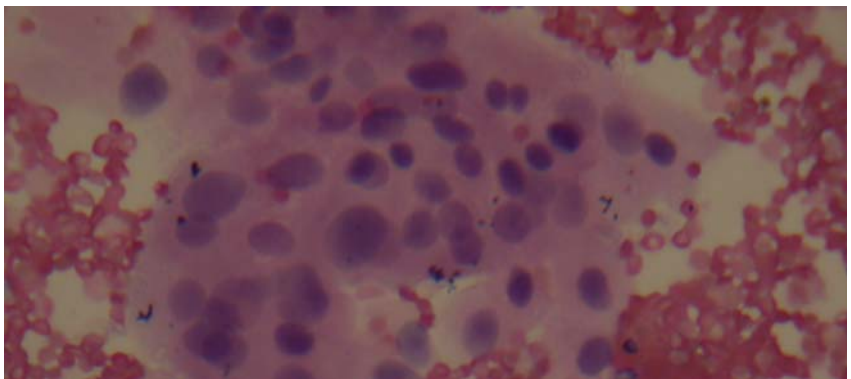
and small cell carcinoma, but lower for other poorly differentiated tumors, because of the overlap of cytomorphologic features of these neoplasms. A combination of cytomorphology and immunocytochemical stains is highly effective in differentiating primary lung carcinoma from metastatic neoplasms.

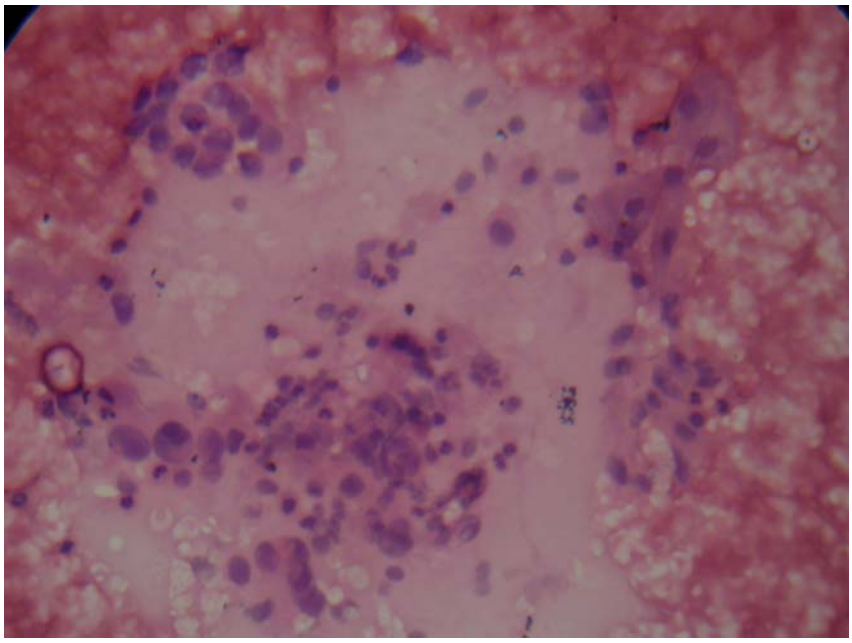
From the results of our study, we conclude that pulmonary cytologic techniques have excellent sensitivity and accuracy in the diagnosis of lung carcinomas. They may establish the diagnosis of lung cancer when endoscopic biopsies give negative results.

Hence we recommend that a combination of the three diagnostic modalities-bronchial washing, brushing and forceps biopsy, is the best strategy in the diagnosis of bronchoscopically visible lung cancer.

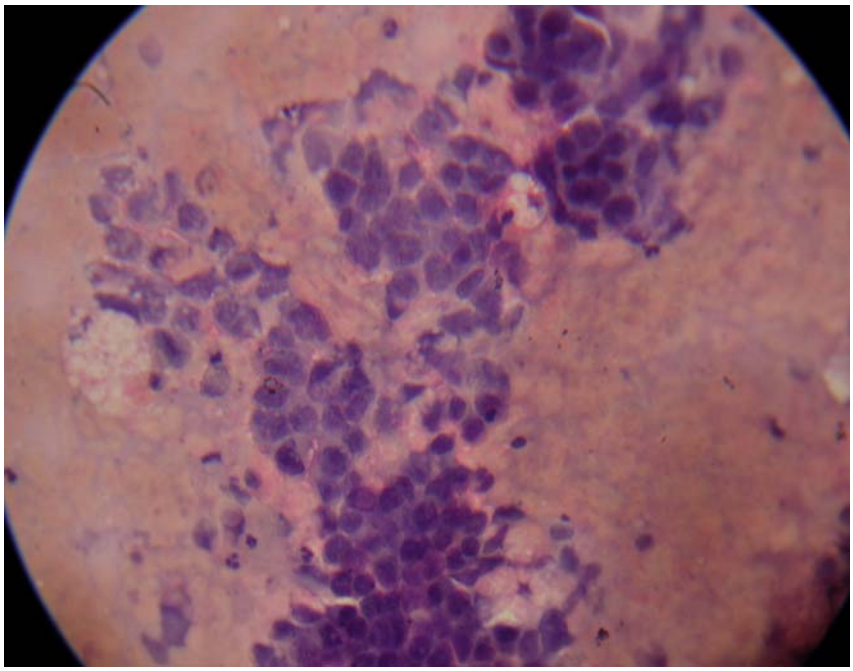
For future studies

All the ancillary studies that are performed on tissue samples (histochemical stains, immuno cytochemical studies, flow cytometry and molecular tests) can also be done on cytology samples to complement the cytological diagnosis of lung cancer.

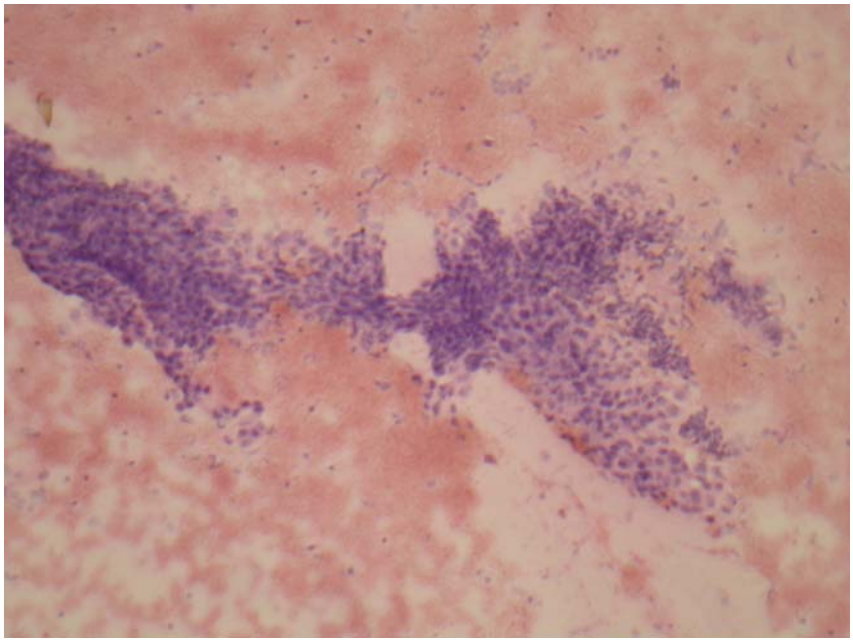




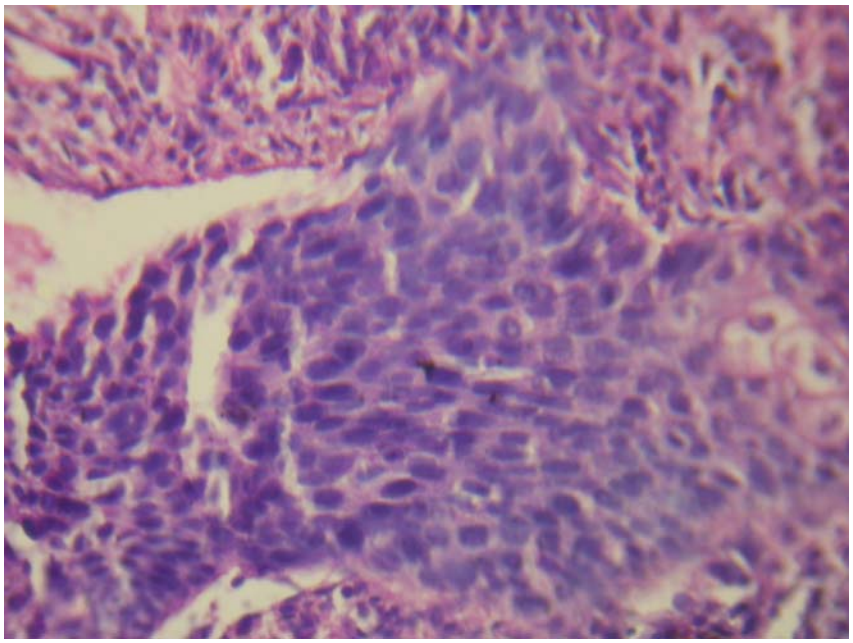
Cy 1044/05-Smear positive for malignancy-Adenocarcinoma (H&E stain x 400x) No biopsy correlation



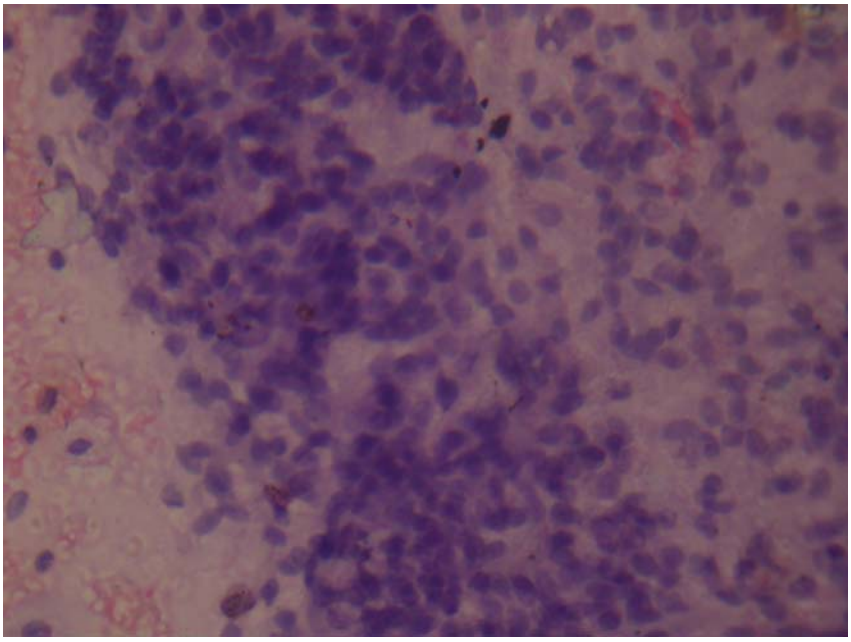
Cy 854/05-Smear positive for malignancy (H&E stain x 400x)



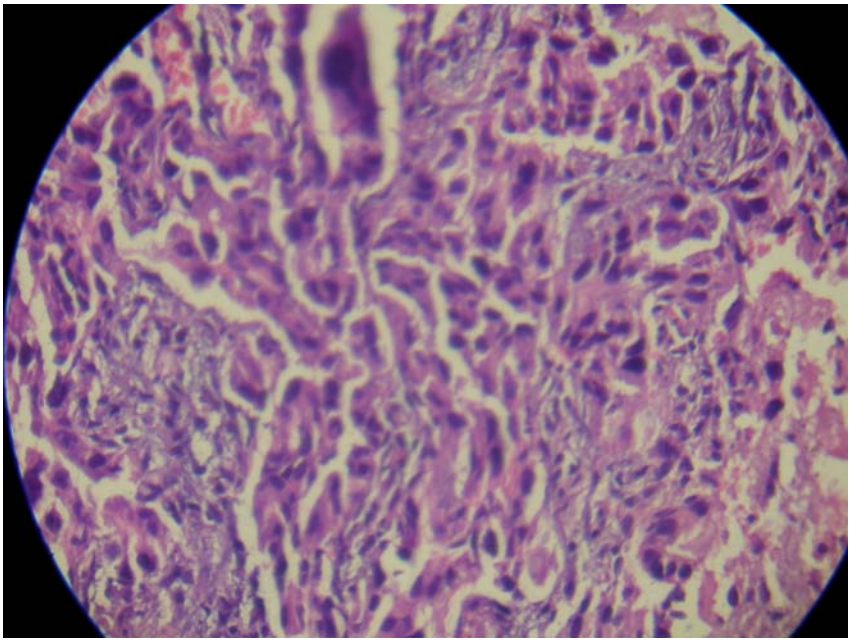
Cy1096/05-Smear suspicious of malignancy (H&E stain x 100x)



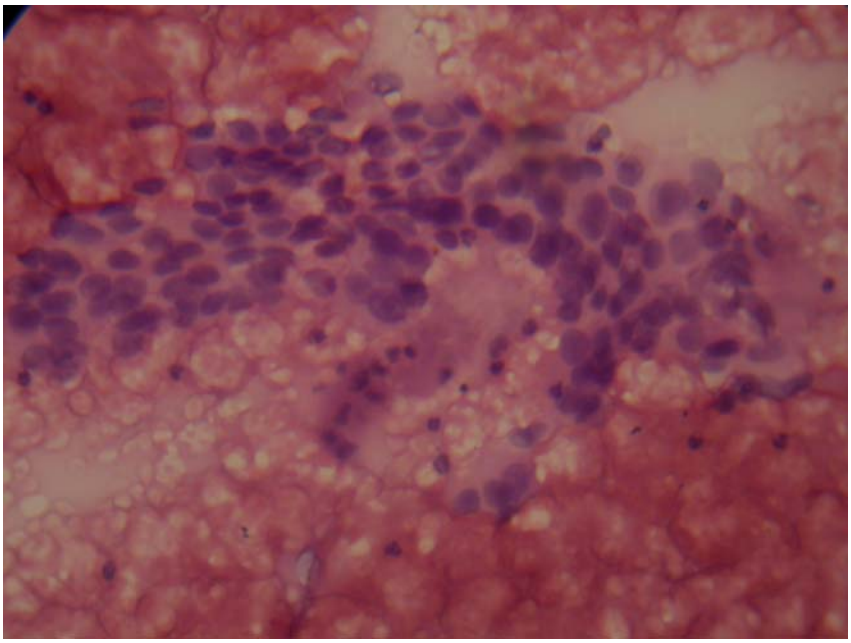
HPE 4094/05-Squamous cell carcinoma (H&E stain x 400x)



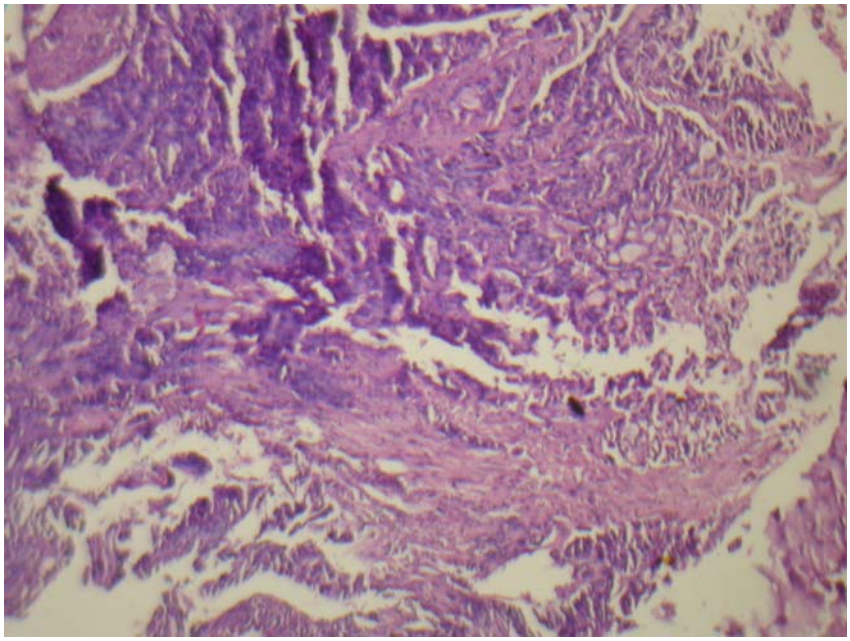
Cy38/05-Smear positive for malignancy (H&E stain x 400x)



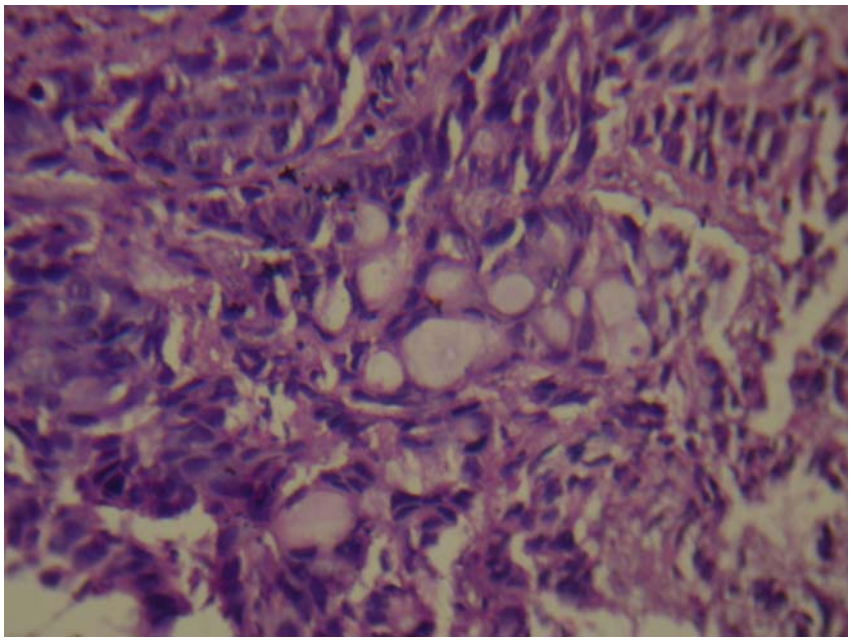
HPE-759/05-Squamous cell carcinoma (H&E stain x 400x)



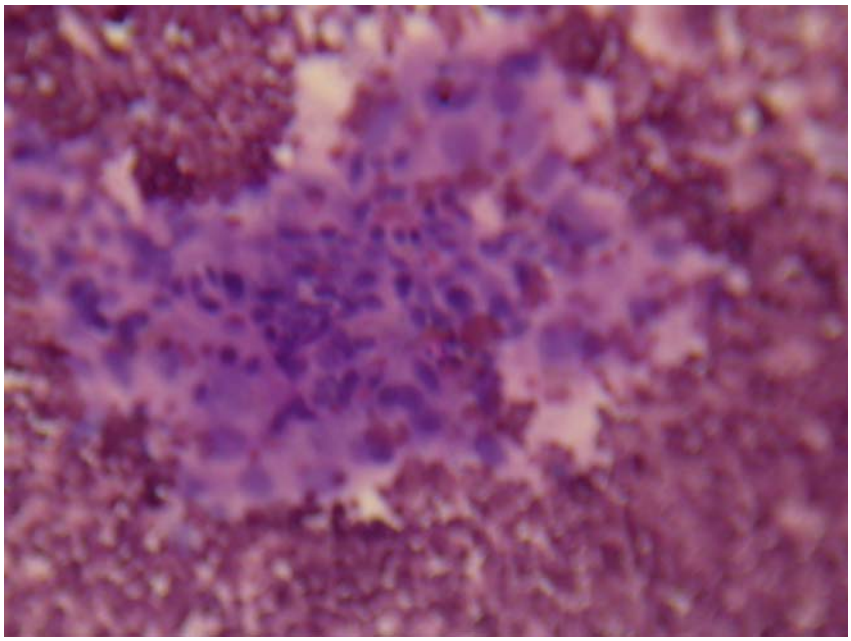
Cy88/05-Smear positive for malignancy-Adenocarcinoma
(H&E stain x 400x)



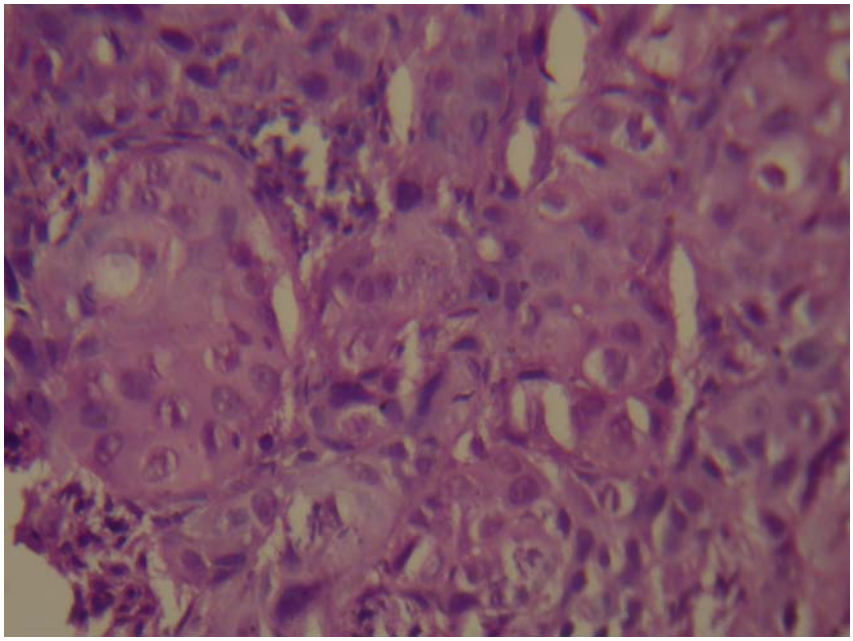
HPE-3316/05-Adenocarcinoma (H&E stain x 100x)



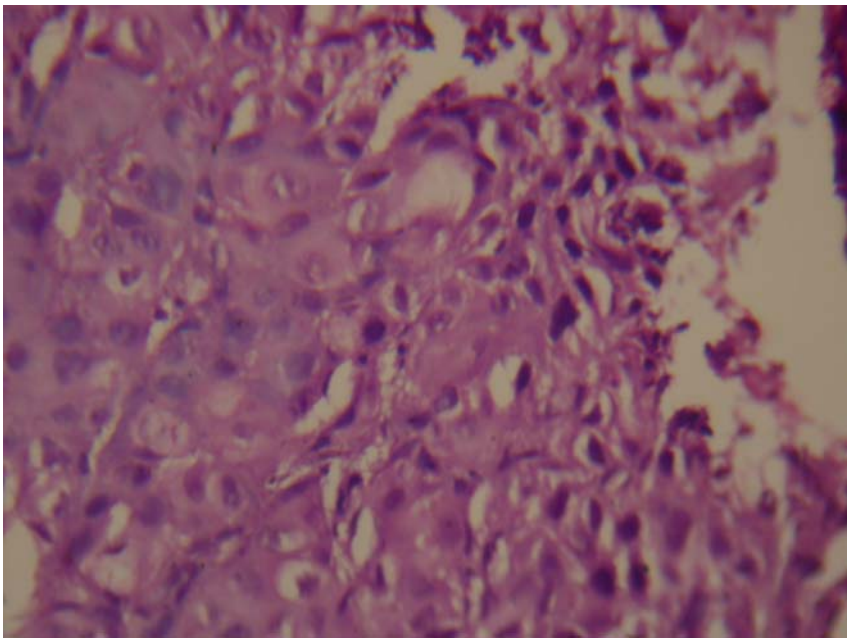
HPE-3316/05-Adenocarcinoma (H&E stain x 400x)



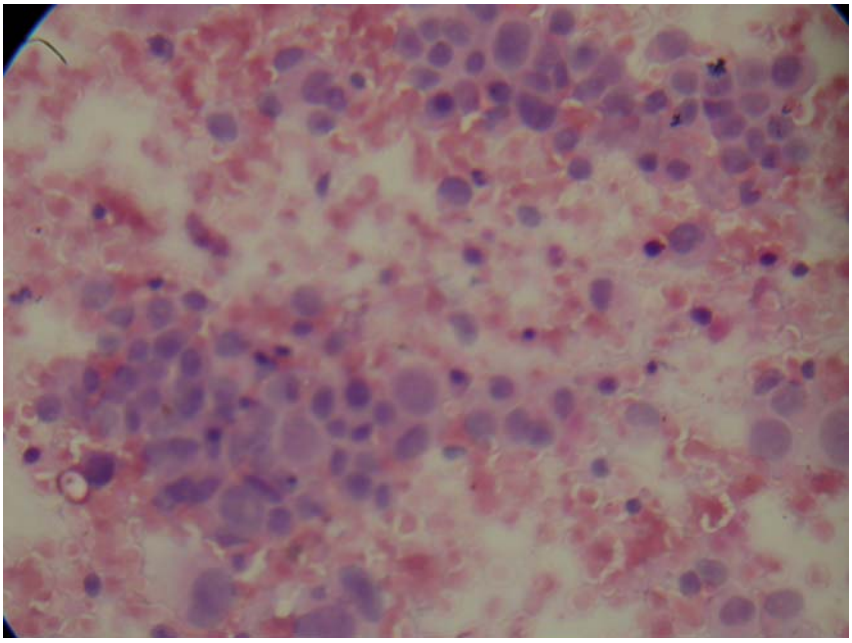
Cy98/05-Smear positive for malignancy (H&E stain x 400x)



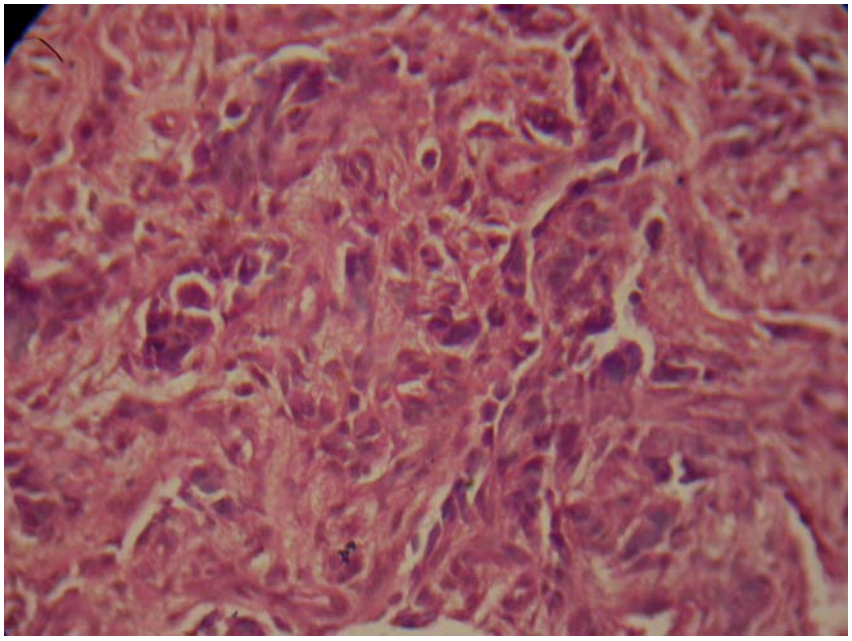
HPE-338/05-Moderately differentiated Squamous cell carcinoma (H&E stain x 400x)



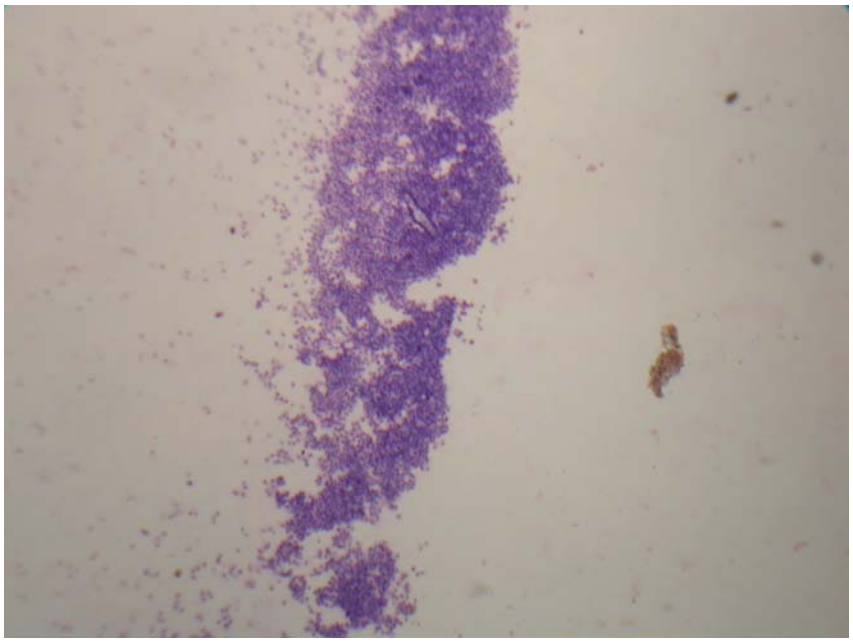
HPE-338/05-Moderately differentiated Squamous cell carcinoma (H&E stain x 400x)



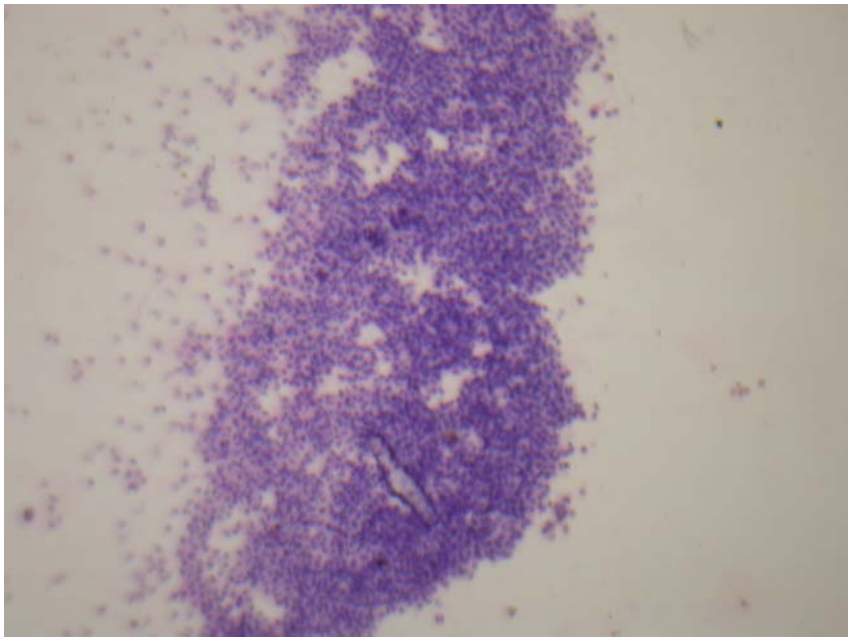
Cy76/05- Smear positive for malignancy- (H&E stain x 400x)



HPE-214/05-Poorly differentiated carcinoma
(H&E stain x 400x)

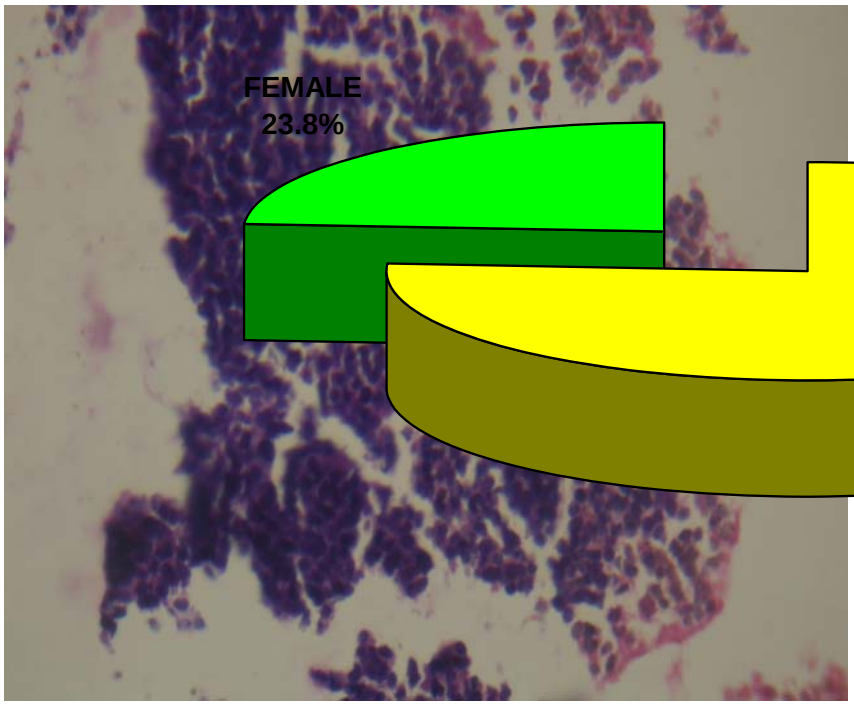


Cy138/05-Smear positive for malignancy-Small cell carcinoma (H&E stain x 50x)



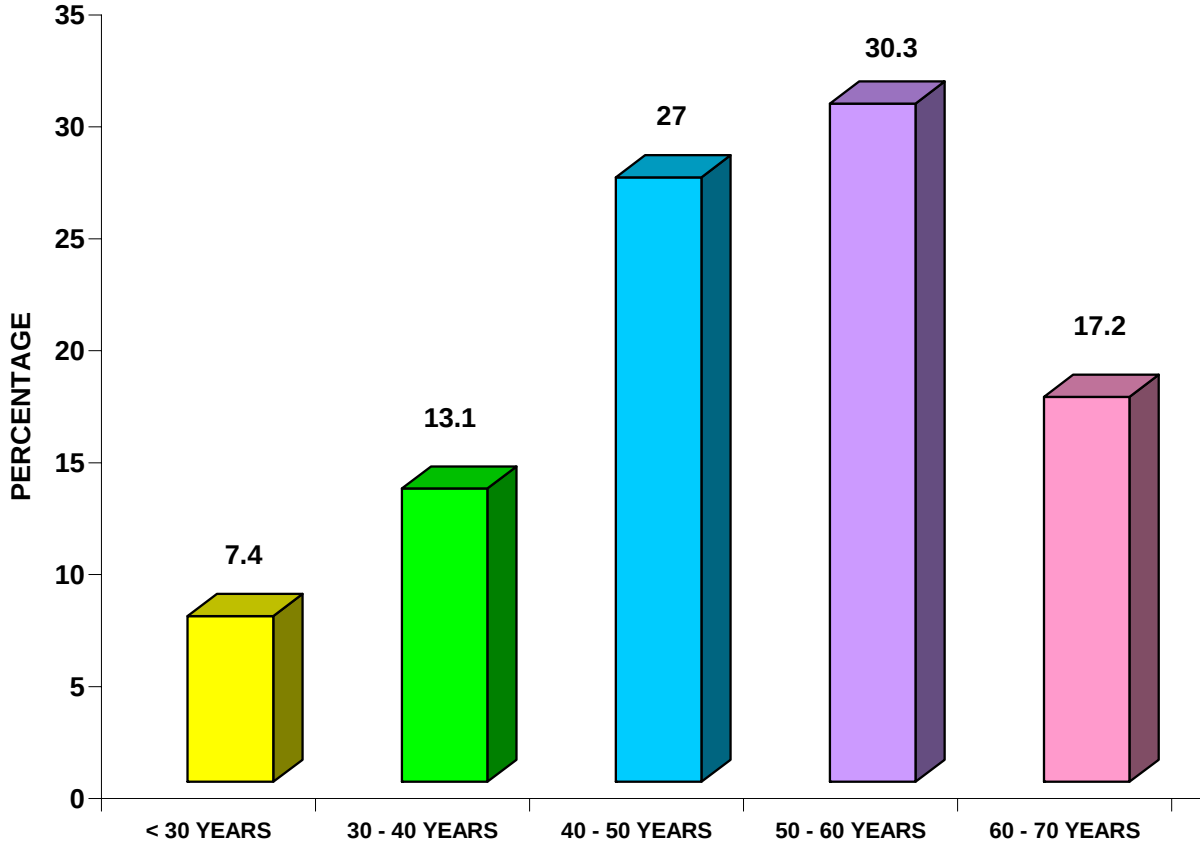
Cy138/05- Smear positive for malignancy-Small cell carcinoma (H&E stain x 100x)

Figure 1: Distribution of Sex



HPE-473/05 Small cell carcinoma (H&E stain x 400x)

Figure 2: Distribution of Age



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